

REMARKS

Claims 39-53 stand rejected under 35 USC §112, first paragraph, as failing to comply with the written description requirement.

A Declaration of Dr. Carl Falco, one of the co-inventors of the above-identified application, was previously submitted on August 24, 2000. The original version of Dr. Falco's Declaration can be found in the file of Application No. 08/823,771 (Attorney Docket No. BB-1037-D).

Submitted herewith, for the Examiner's convenience, is another copy of a Declaration of Dr. Carl Falco dated August 24, 2000. Dr. Falco's Declaration dated August 24, 2000, shows that with the *Arabidopsis* LKR/SDH fragments in hand, it was possible to isolate LKR/SDH fragments from any other plant desired, and use them to block expression utilizing antisense inhibition and/or cosuppression. Dr. Falco's Declaration demonstrated that blocking the first step in lysine catabolism, i.e., "knocking out" LKR/SDH, leads to increased accumulation of lysine in seeds. It is stated specifically in paragraph 9 of Dr. Falco's August 24, 2000 declaration that :

9. The corn LKR/SDH cDNA sequence was used to identify transposon mutations in the endogenous corn LKR/SDH gene via PCR screening of a library of corn lines containing Robertson's Mutator transposon insertions. The precise location of Mutator insertions into the LKR/SDH gene was determined by sequencing of genomic DNA from individual mutants. An insertion mutation located in an exon in the LKR domain of the gene was chosen for further study. Southern blot analysis of corn genomic DNA indicated that corn contains only one LKR/SDH gene. Since an insertion mutation is expected to block function of the gene, it was anticipated that such a mutation would be recessive. One fourth of the progeny seed from a selfed corn ear with such a mutation segregating would be expected to be homozygous for the mutation. It was observed that approximately one fourth of such seed exhibited a higher level of free lysine than normal (5 to 15 fold higher) without the increase in the lysine catabolite saccharopine that is seen when free lysine is increased via expression of lysine insensitive DHDPS. It was concluded that knocking out LKR/SDH, by itself, was able to increase seed lysine content in corn seeds.

The LKR/SDH Mutator insertion line was crossed by a transgenic line that accumulates excess free lysine due to expression of lysine insensitive DHDPS and AK. In this cross two genetic loci that affect lysine accumulation, one of which is recessive (the LKR/SDH Mutator insertion) and one of which is semi-dominant (the lysine insensitive DHDPS and AK transgene locus), are segregating. Single seeds were analyzed for lysine and saccharopine content. The most striking observation from this experiment is that the highest lysine containing seeds have low levels of saccharopine (see figure). The low saccharopine level indicates that these seeds are homozygous for the LKR/SDH Mutator insertion, while the high lysine level indicates that

they carry the lysine insensitive DHDPS and AK transgene locus. The level of lysine accumulation is considerably higher (2-3 fold) than the level provided by the DHDPS and AK transgene locus alone. Thus, this experiment demonstrates that an increase in the accumulation of lysine, accompanied by a reduction in accumulation of lysine catabolites can be accomplished by combination of lysine overproduction brought about by expression of lysine insensitive DHDPS + AK and reduction of lysine catabolism by blocking expression of LKR/SDH, as we taught in the patent application. These results show that the concern stated in the Office Action on page 5 that "modifying metabolic pathways ... is highly unpredictable and often the desirable results are impossible to achieve" is unfounded in this particular case.

As indicated above, LKR/SDH expression has been blocked in corn via cosuppression. To accomplish this a chimeric gene designed for cosuppression of LKR was constructed by linking a 1268 bp LKR/SDH gene fragment, which included the LKR coding domain, to the corn endosperm 27 kD zein promoter and 10 kD zein 3' untranslated region. This chimeric gene was introduced into corn by particle-gun mediated transformation. Over 100 transformed lines were obtained. Of 72 transformation events that were regenerated into plants and produced seed, 13 had seeds with a greater than four fold increase in free lysine. This is a typical frequency for cosuppression events. Since the transformed plants were out-crossed, the transgenic locus must be dominant or there would not have been any observable phenotype. This is expected from a cosuppression transgene, and is an advantage over knock-out mutations like the LKR/SDH Mutator insertion described above.

Some of the LKR cosuppression transformants have been carried forward for further testing. An event that has continued to show the increased free lysine phenotype for several generations and behaves genetically as a single locus transgene insertion has been selected for crossing to the transgenic line that accumulates excess free lysine due to expression of lysine insensitive DHDPS and AK. Results from that experiment are not yet available, but the expectation is that seeds carrying both transgene loci will have higher lysine levels than either parent, as was observed in the LKR Mutator insertion cross described above. In addition, co-transformation experiments in which the chimeric gene designed for cosuppression of LKR described above has been combined with a chimeric gene for expression of lysine insensitive DHDPS and introduced into corn by particle-gun mediated transformation are in progress. This is expected to yield transformants that produce seeds with the high lysine level observed in the LKR Mutator insertion cross by lysine insensitive DHDPS and AK, but with both chimeric genes at a single genetic locus, which is highly desirable for corn breeding.

Also submitted herewith is a copy of a second Declaration of Dr. Carl Falco, (the original version can be found in the file of Application No. 08/823,771 (Attorney Docket No. BB-1037-D)). Dr. Falco's Declaration dated

February 16, 2001, sets forth data showing seeds with increased lysine that were obtained from plants co-transformed with DHDPS and LKR.

The experiments discussed in Dr. Falco's Declaration dated February 16, 2001 along with the information submitted previously and the detailed description of the invention provided in the instant application demonstrate that seeds having an increased lysine content can be made when a lysine insensitive DHDPS gene (with or without a lysine insensitive AK gene) is combined with a co-suppressing LKR gene.

This coupled with the remarks presented previously in the Response and accompanying Attachments A-D all of which were electronically filed on July 20, 2007 are believed to address the issues raised with respect to what structural features would confer either type of enzyme activity as well as addressing what sequences would be sufficient for use in antisense inhibition or sense suppression of LKR/SDH.

Accordingly, withdrawal of the rejection of claims 39-53 under 35 USC §112, first paragraph, as failing to comply with the written description requirement, is respectfully requested in view of the foregoing discussion and attachments.

Claims 39-53 stand rejected under 35 USC §112, first paragraph, as failing to comply with the enablement requirement.

It is respectfully submitted that the foregoing discussion pertaining to the written description rejection is equally apposite with respect to the this ground of rejection.

Thus, withdrawal of the rejection of claims 39-53 under 35 USC §112, first paragraph, as failing to comply with the enablement requirement, is respectfully requested in view of the foregoing discussion and attachments.

A petition for a two (2) month extension of time accompanies this Response After Final along with copies of the above-identified previously submitted declarations of Dr. Carl Falco.

A Notice of Appeal also accompanies this Response After Final.

It is respectfully submitted that the application is in form for allowance which allowance is respectfully requested.

The Commissioner is authorized to charge Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company) for any fees associated with the filing of this response.

In view of the foregoing, allowance of the above-referenced application is respectfully requested.

Respectfully submitted,

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